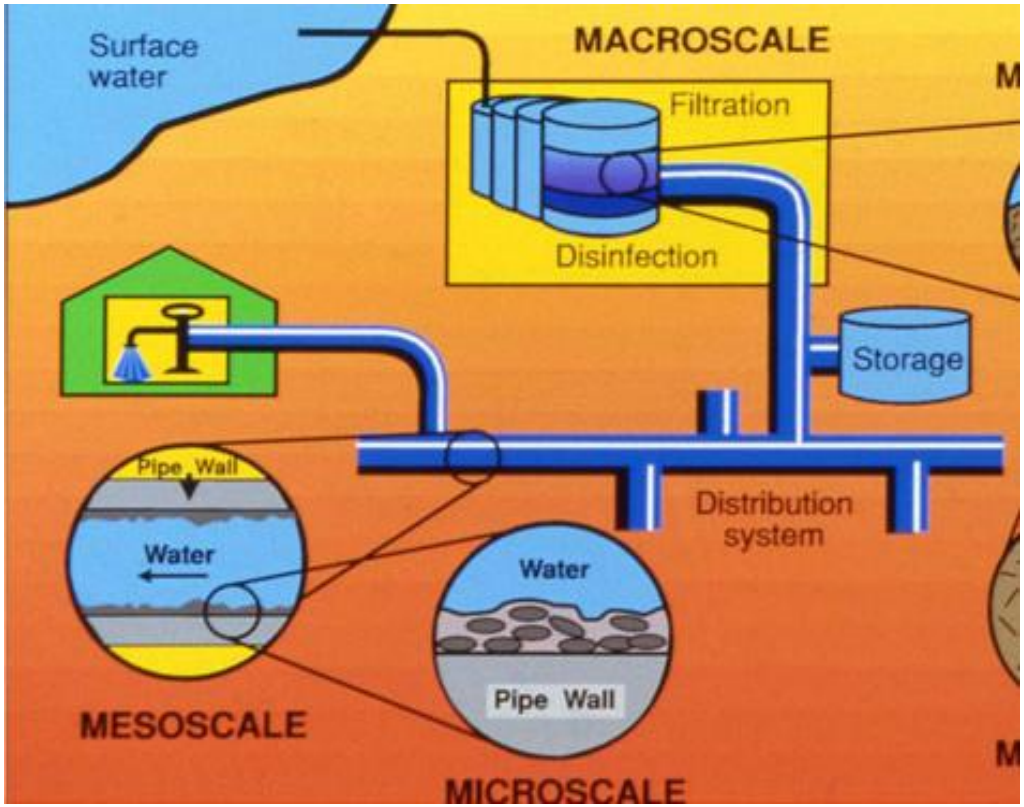
Pathogen Association with Drinking Water Distribution Systems

T. H. (Helen) Nguyen and W.T. Liu, University of Illinois at Urbana-Champaign
R. M. Hozalski, Univ. of Minnesota

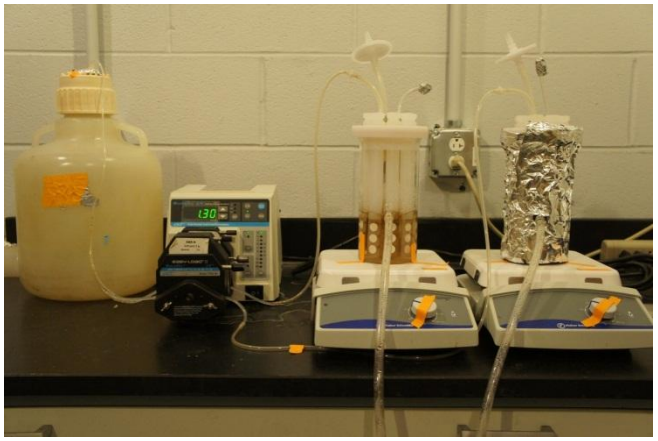


Project Objectives

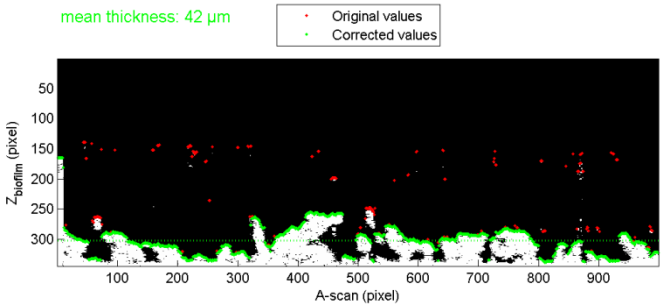
- To identify the factors that influence the persistence of a model pathogen, *Legionella*, in multi-species biofilms grown on different pipe materials (Illinois).
- To characterize the microbial communities and the surface properties of the biofilms grown on different pipe materials and chlorine level (both Illinois and Minnesota).
- To further investigate novel chemical and enzymatic treatments to weaken the biofilm to promote detachment and improved cleaning (Minnesota).

Methodology (Illinois)

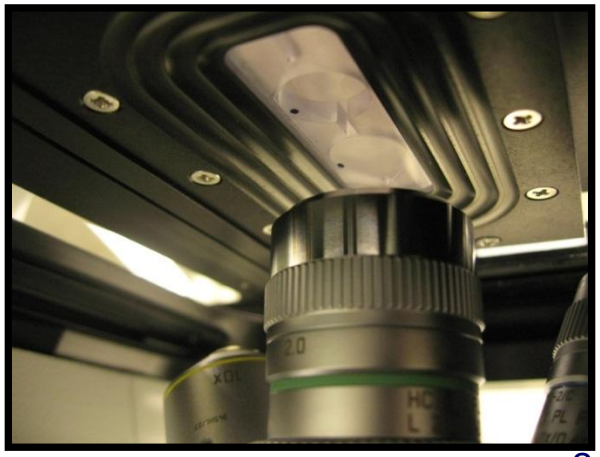
Biofilms are grown in CDC reactors using groundwater, tap water and dechlorinated tap water



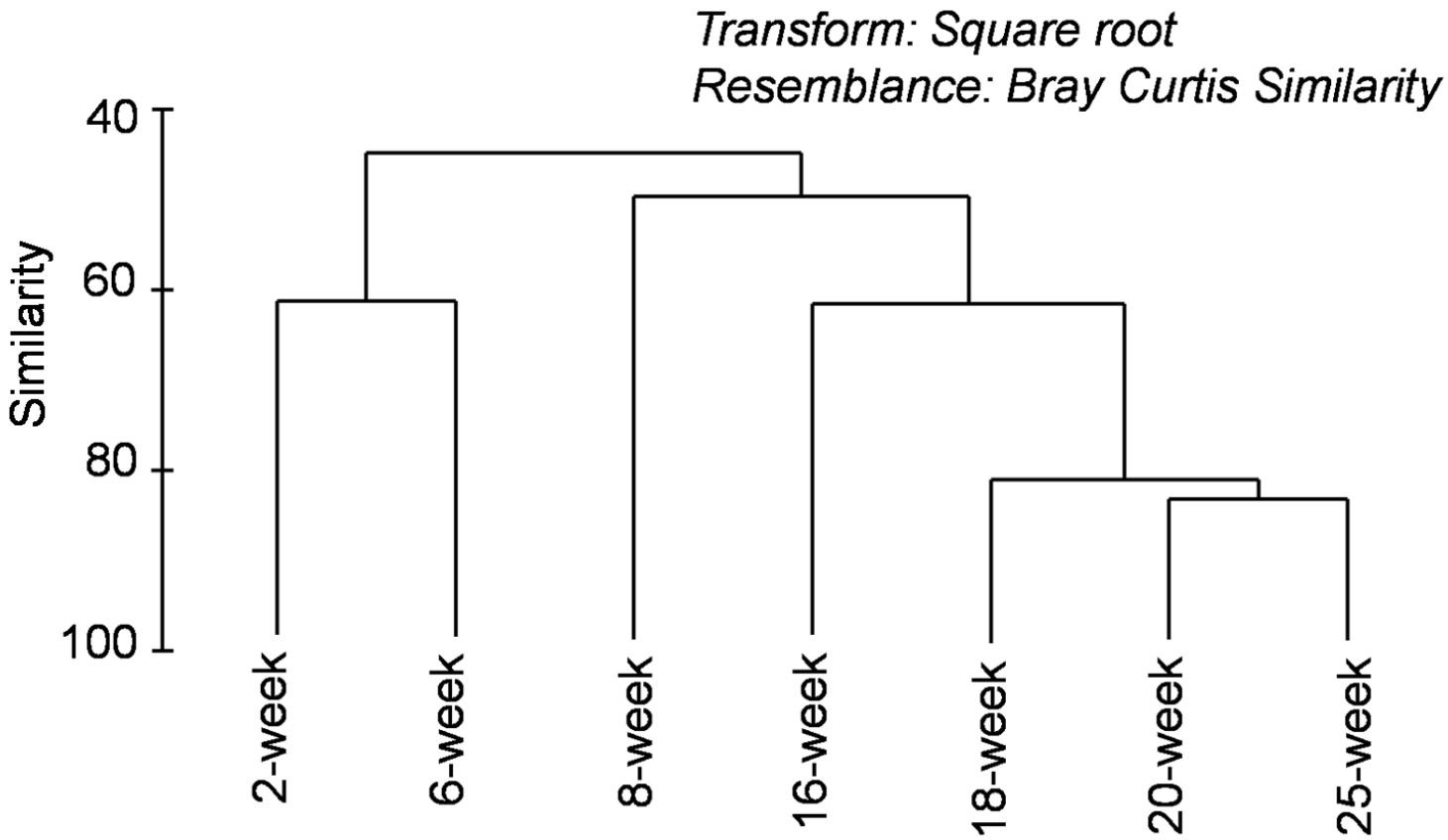
Biofilm analysis



E. coli or Legionella deposition

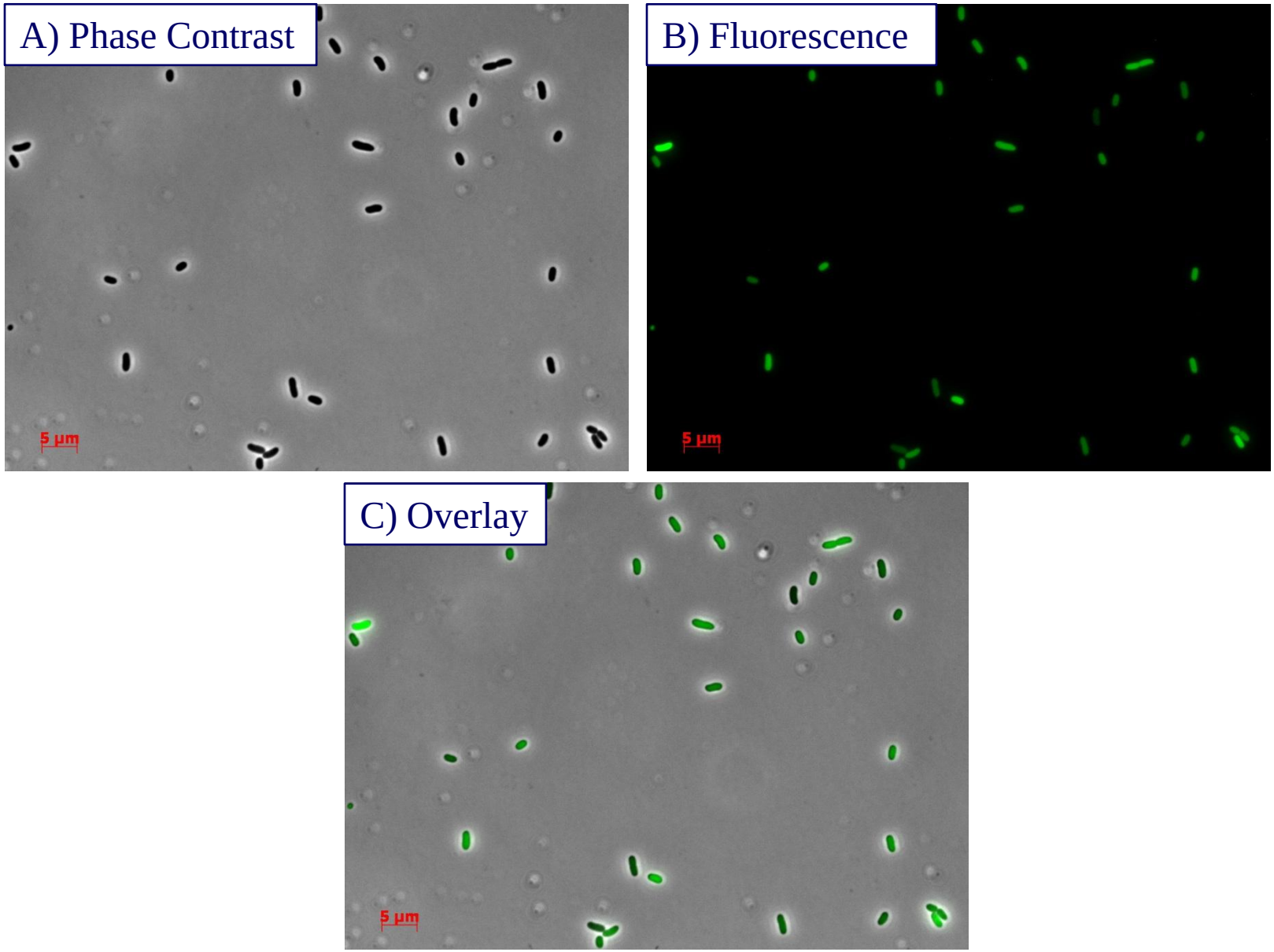


Microbial community analysis using Terminal Restricted Fragment Length Polymorphism (TRFLP)showed relatively stable biofilm after 16 weeks

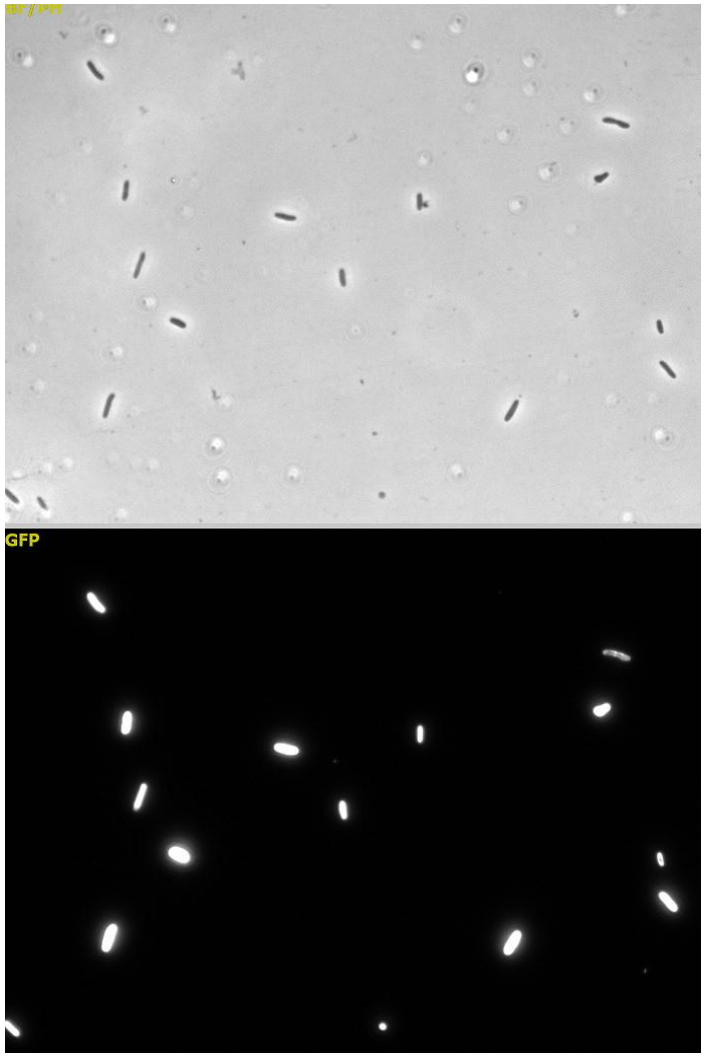


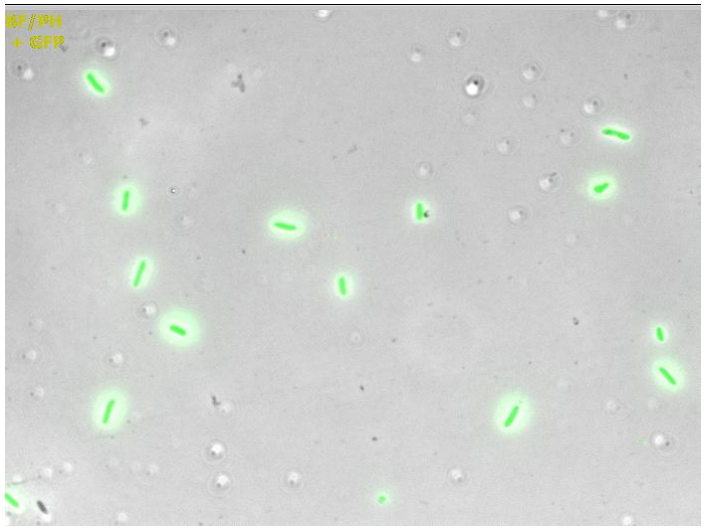
Janjaroen et al., 2013, Water Research, in press

gfp-tagged environmental *E. coli* is used in deposition experiment.

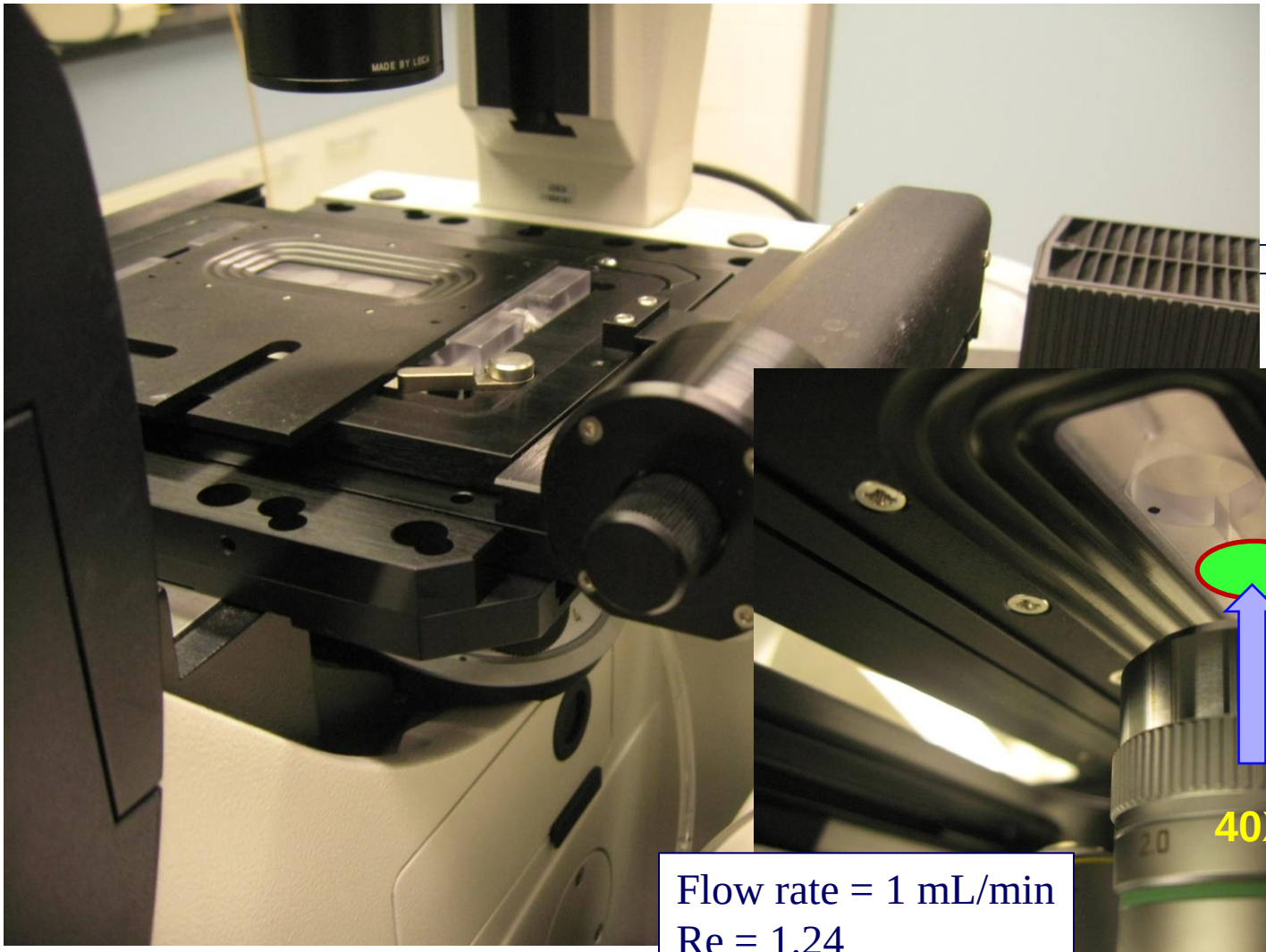


Legionella pneumophila (ATCC33152) tagged with GFP plasmid pBG307 (Chen et al., Plasmid, 2006)





Parallel plate flow cells are used to observe *E. coli or Legionella* deposition on PVC and biofilms.



KCl solution
pH 8.2-8.5
3-300 mM

Flow rate = 1 mL/min
Re = 1.24

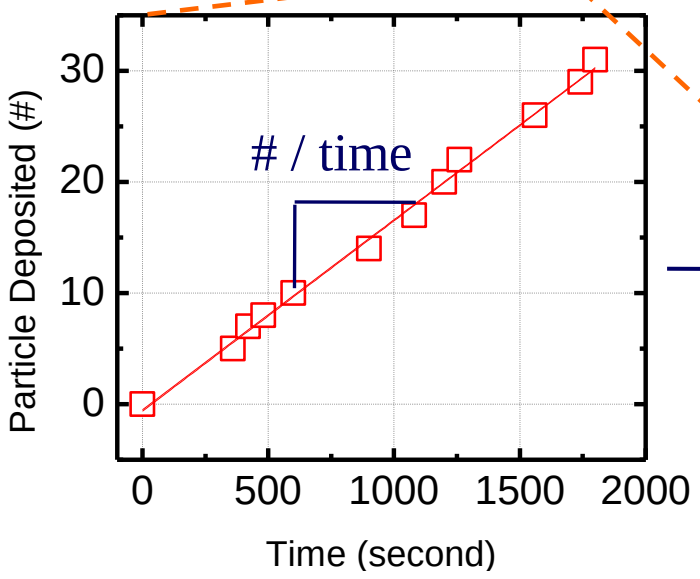
Sherwood number calculation for *E. coli* or *Legionella* adhesion to biofilm surface

Deposition rate coefficient (k_D)(m/s)

$$k_D = \frac{J}{C_0}$$

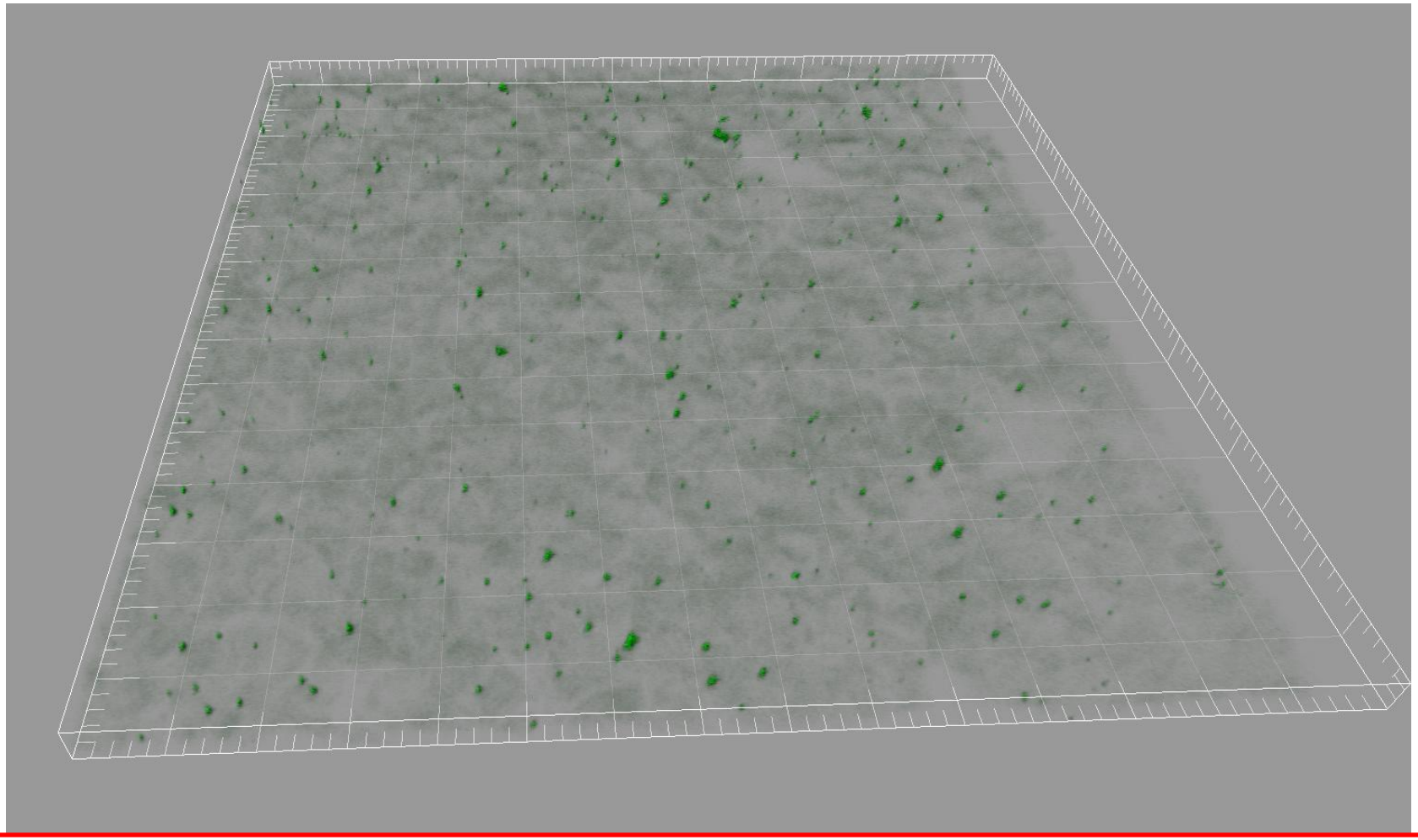
Sherwood number

$$Sh = \frac{J \times a}{C_0 \times D}$$



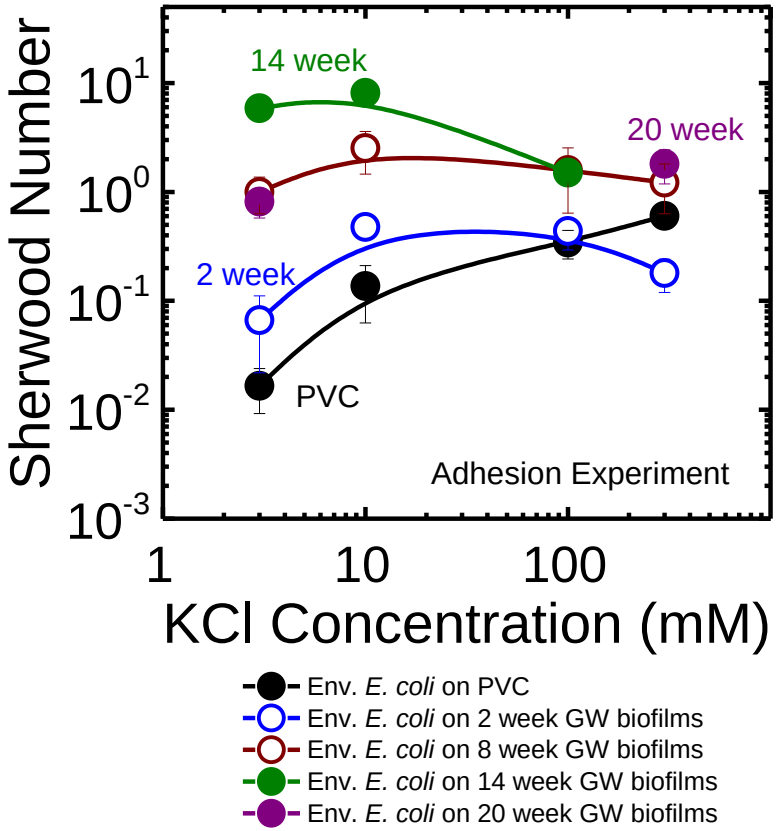
Normalized by viewing area (296 X 222 μm)

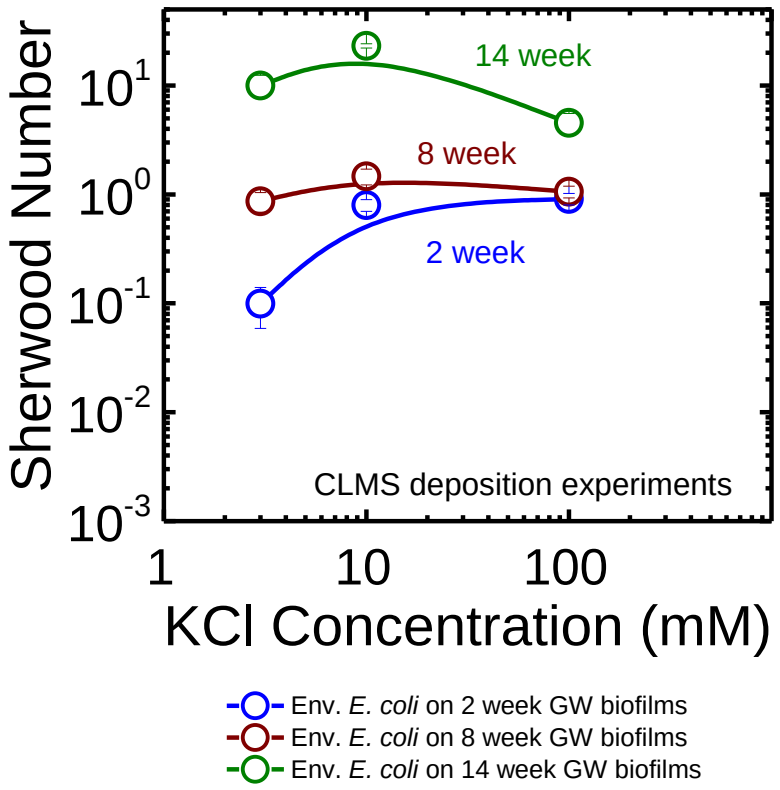
a= bacteria diameter (m)
D = diffusion coefficient (m^2/s)



Sherwood number

$$Sh = \frac{J \times a}{C_0 \times D}; \quad \text{confocal } J = \frac{N}{A \times t}$$





Clean PVC

2w biofilm

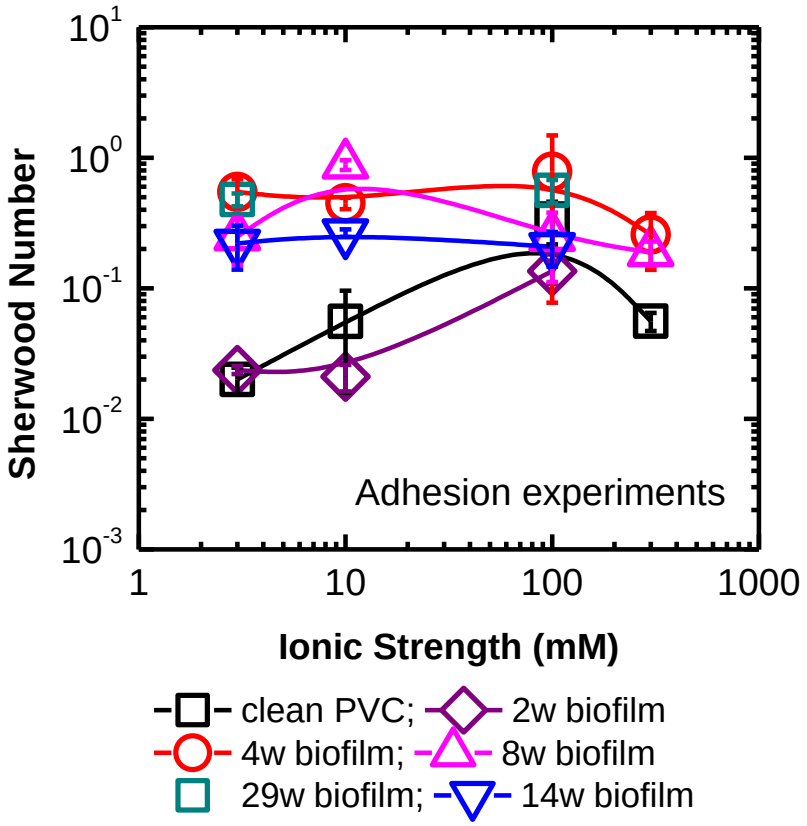
8w biofilm

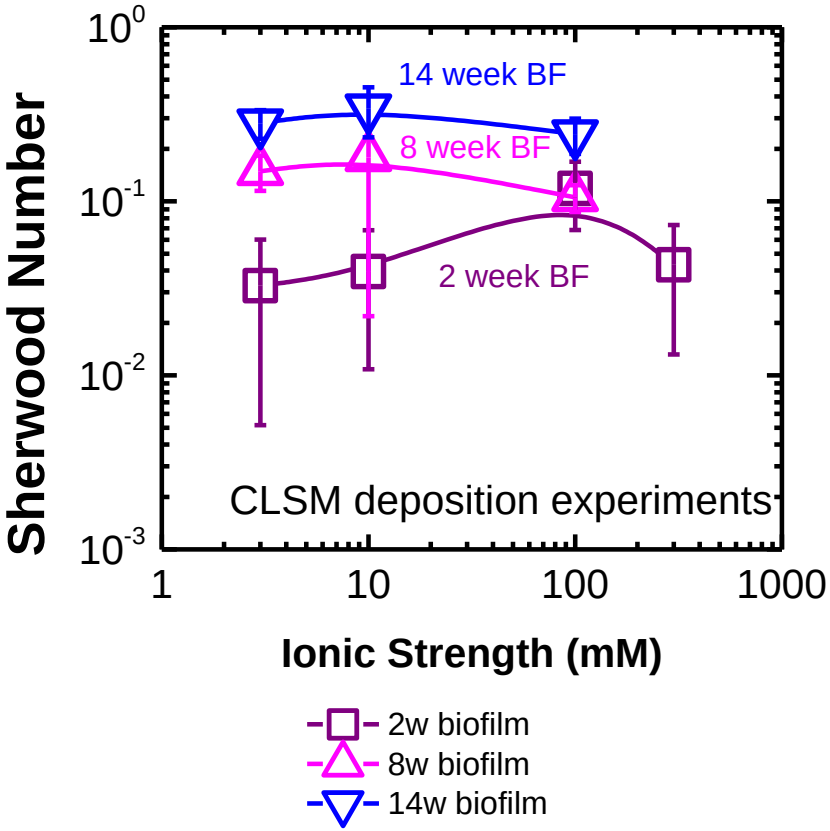
14w biofilm

20w biofilm

- Higher *E coli* deposition on older biofilms compared to on PVC.
- *E coli* deposition on old biofilms did not increase with ionic strength

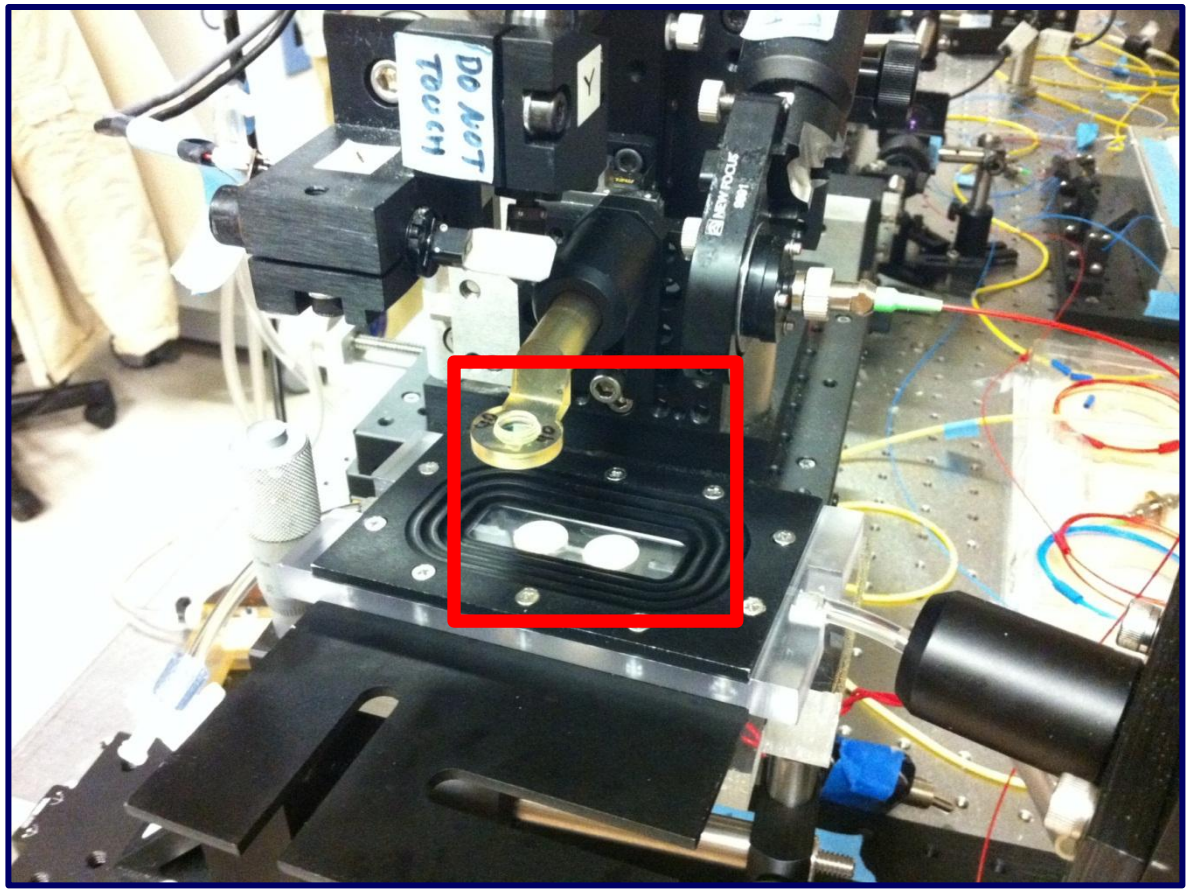
The trend in *Legionella* deposition on biofilms is similar to the *E. coli* trend



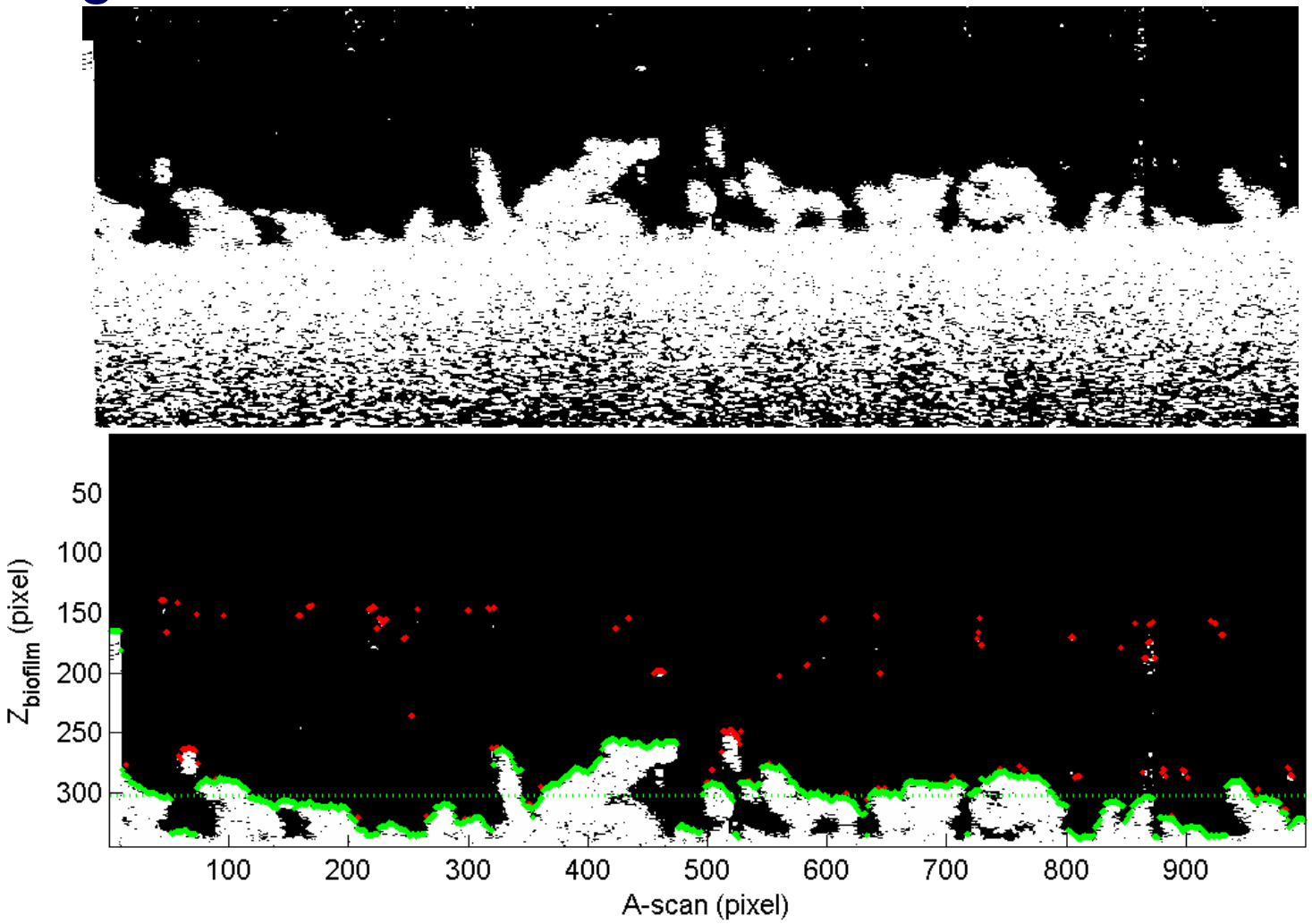


Clean PVC | 2w biofilm | 4w biofilm | 8w biofilm | 12w biofilm | 14w biofilm

Optical coherence tomography (OCT) captures micrometer-resolution, three-dimensional images from within optical scattering media.

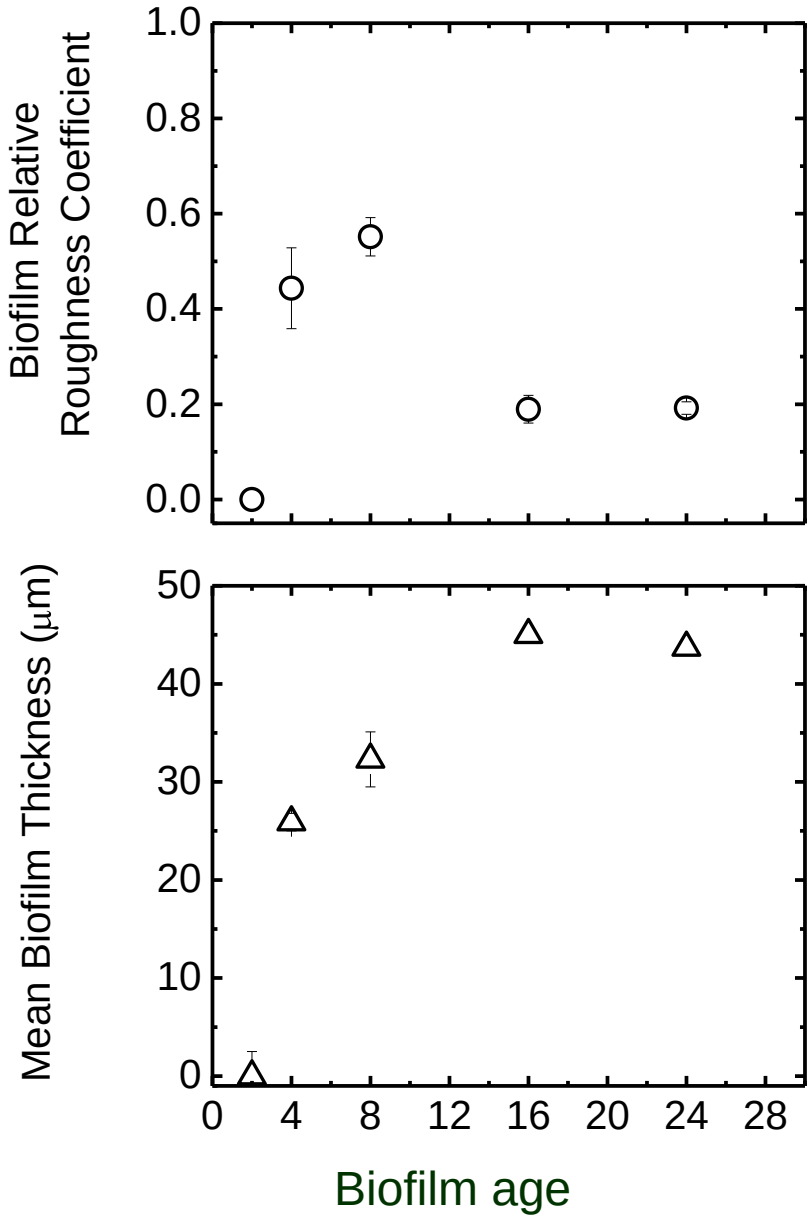


Biofilm surface roughness is calculated from analysis of optical coherence tomography (OCT) images.



Mean thickness 42 μm

Quantitative biofilm characterization



Mean Biofilm Thickness

$$\bar{z} = \frac{1}{n}\sum_{i=1}^{N} z_i$$

Absolute roughness

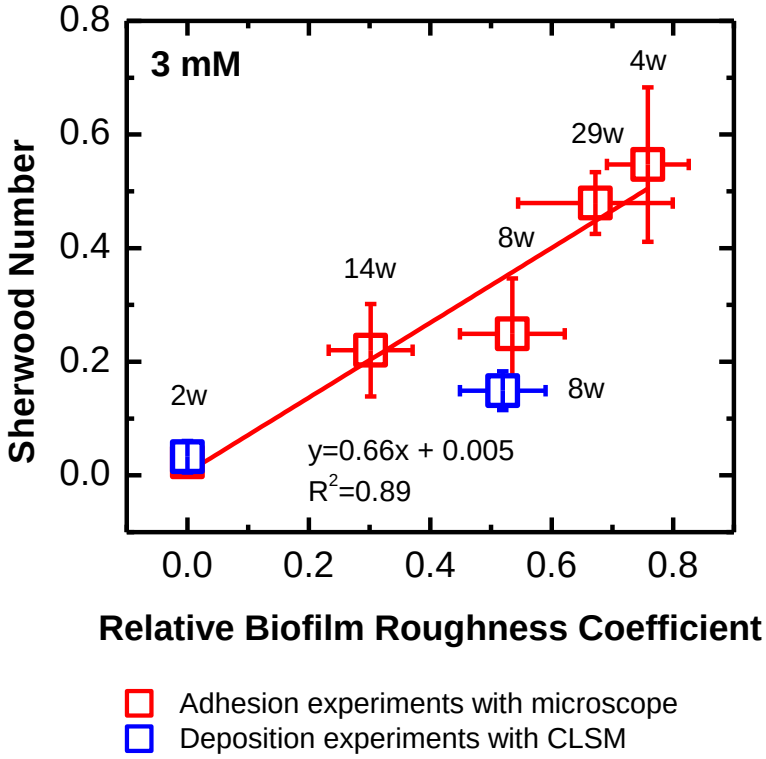
$$R_a = \frac{1}{n}\sum_{i=1}^{N}\left(\left|z_i - \bar{z}\right|\right)$$

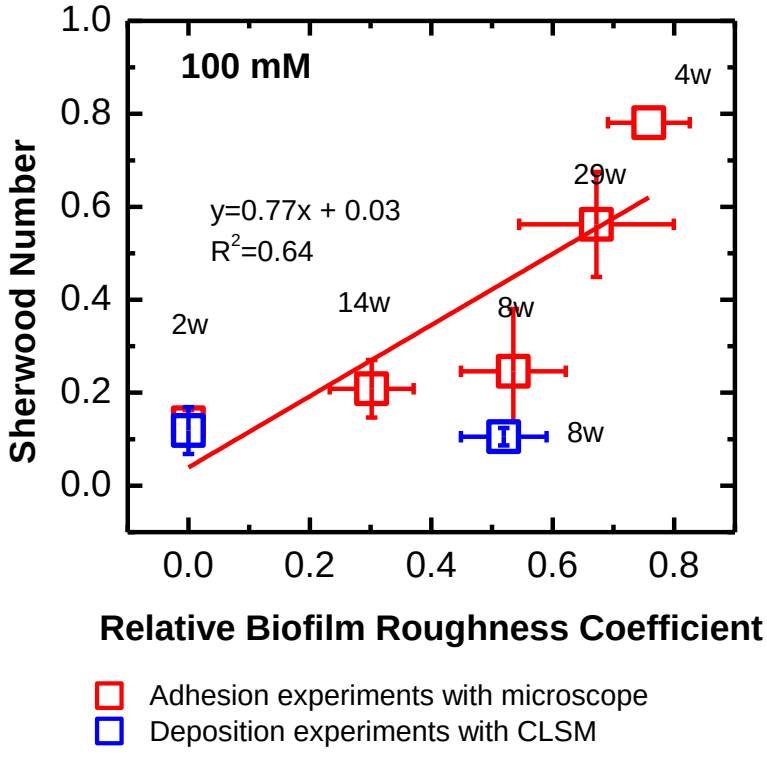
Relative roughness coefficient

$$R_a' = \frac{1}{n}\sum_{i=1}^{N}\left(\frac{\left|z_i - \bar{z}\right|}{z}\right)$$

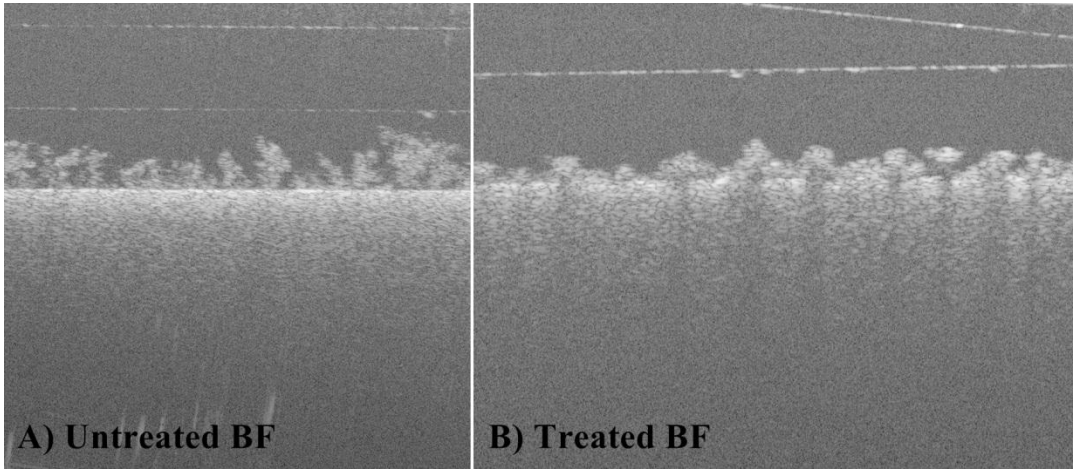
Janjaroen et.al, 2013, Water Research

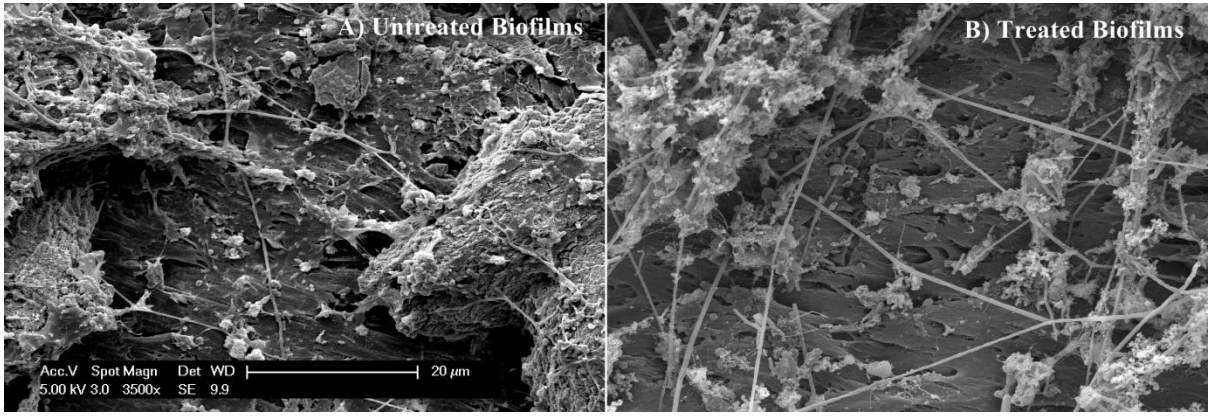
Legionella deposition correlate with biofilm roughness



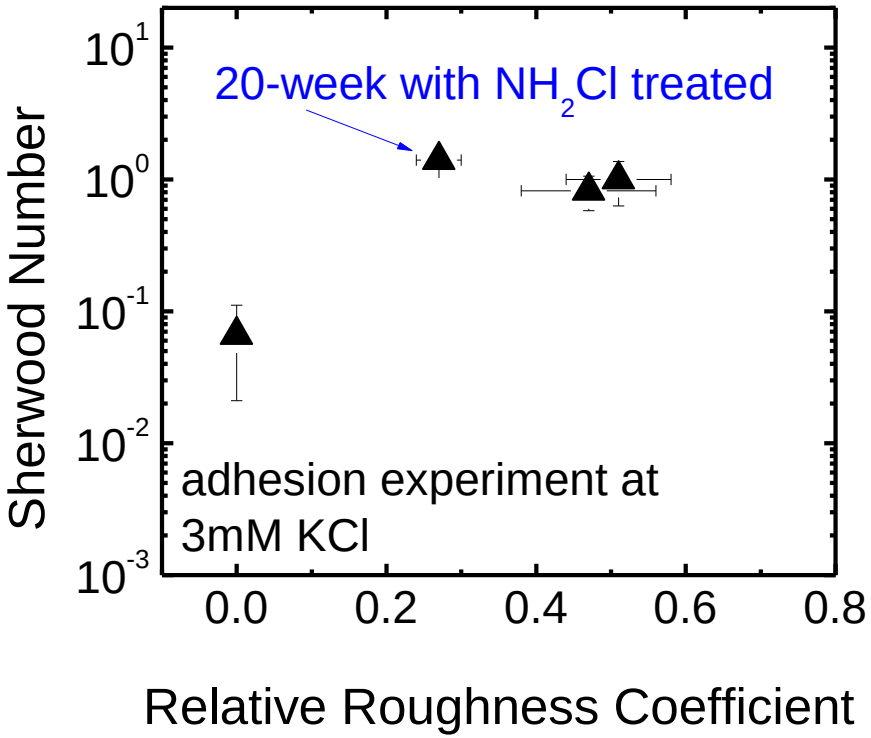


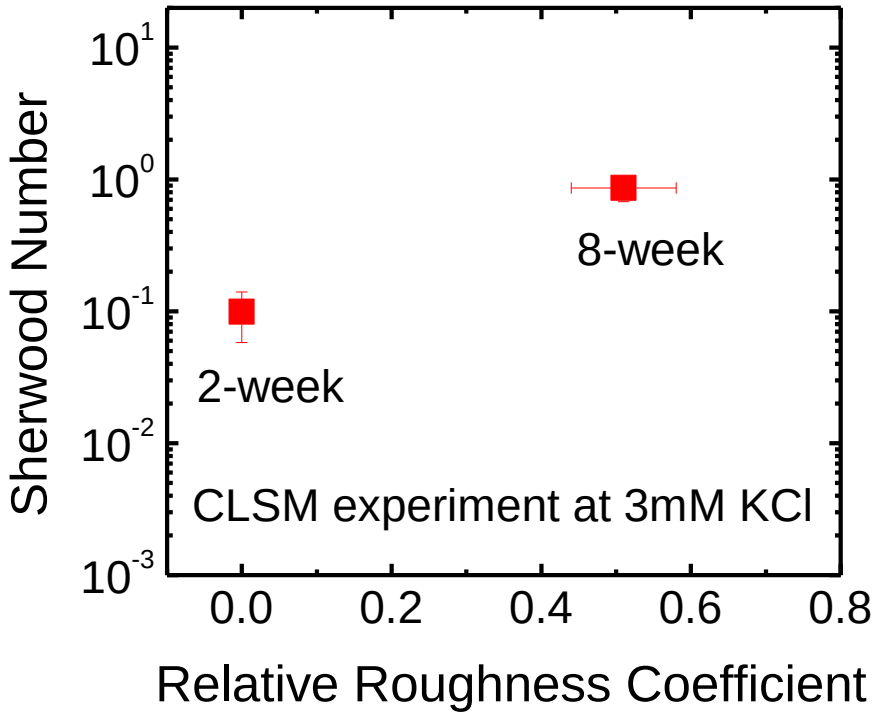
OCT and SEM images show no difference in untreated and chloramine treated biofilms





E. Coli attaches more on rougher biofilm





Conclusion and future work for deposition study

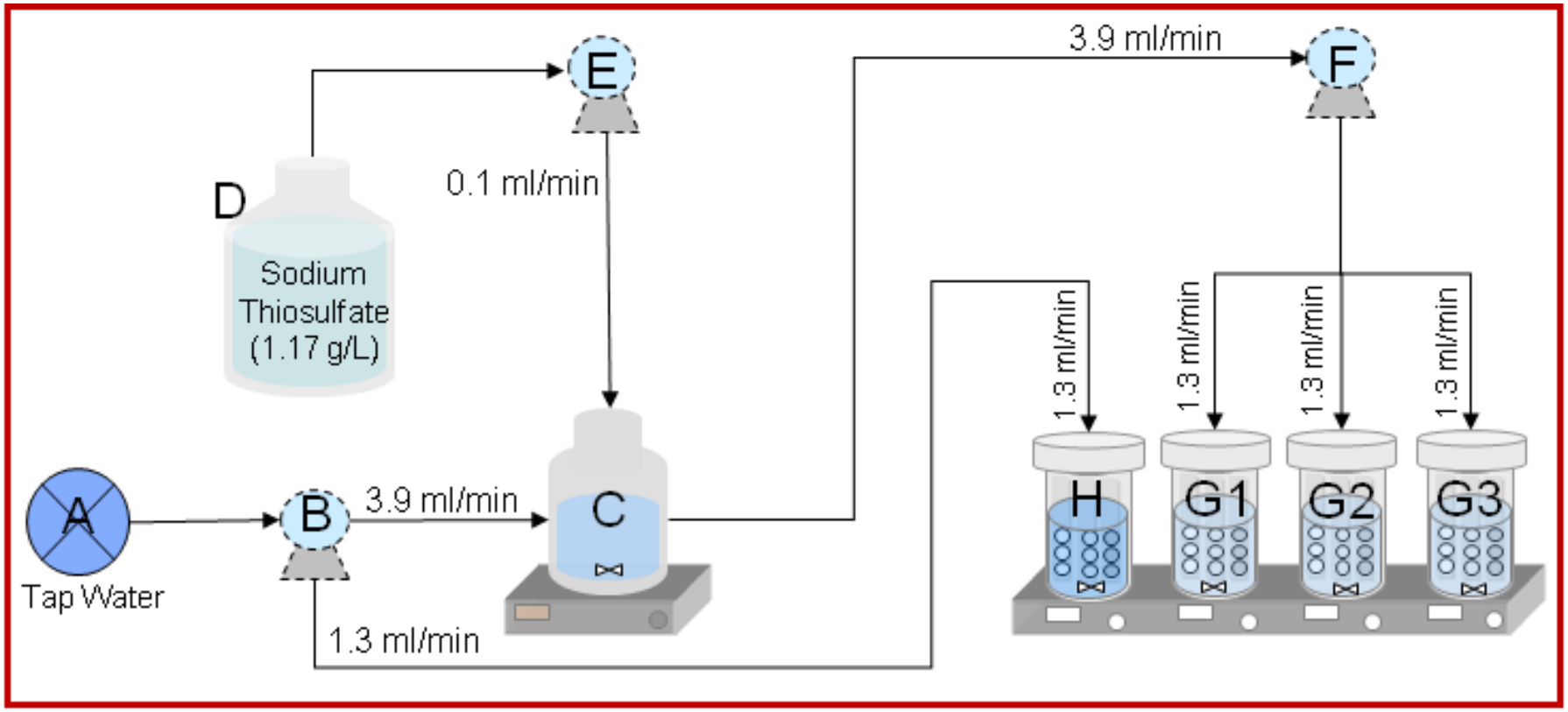
- Higher *Legionella* and *E. coli* deposition on biofilms compared to on PVC surface was observed.
- *Legionella* and *E. coli* deposition is controlled by biofilm roughness not the water ionic concentration.
- 20 week of exposure to chloramine did not change the biofilm roughness. Similar deposition was observed on biofilms with and without exposure to chloramine.

Conclusion and future work for deposition study

- Deposition will be conducted with biofilms grown on concrete coupons and coupons coated with calcite.
- Deposition will be conducted with biofilms treated with biofilm weakening agents.

UMn Tasks

- Investigate effect of pipe material (and chlorine level) on biofilm development, community composition, and detachment/removal
  - CDC Reactors
  - 24 Well Plates
- Test effect of treatments on biofilm weakening and removal
  - 96 Well Plates
  - Metal chelators: polyphosphate, citrate



A: Tap water

B: Pump (3.9 ml/min)

C: Dechlorination reactor

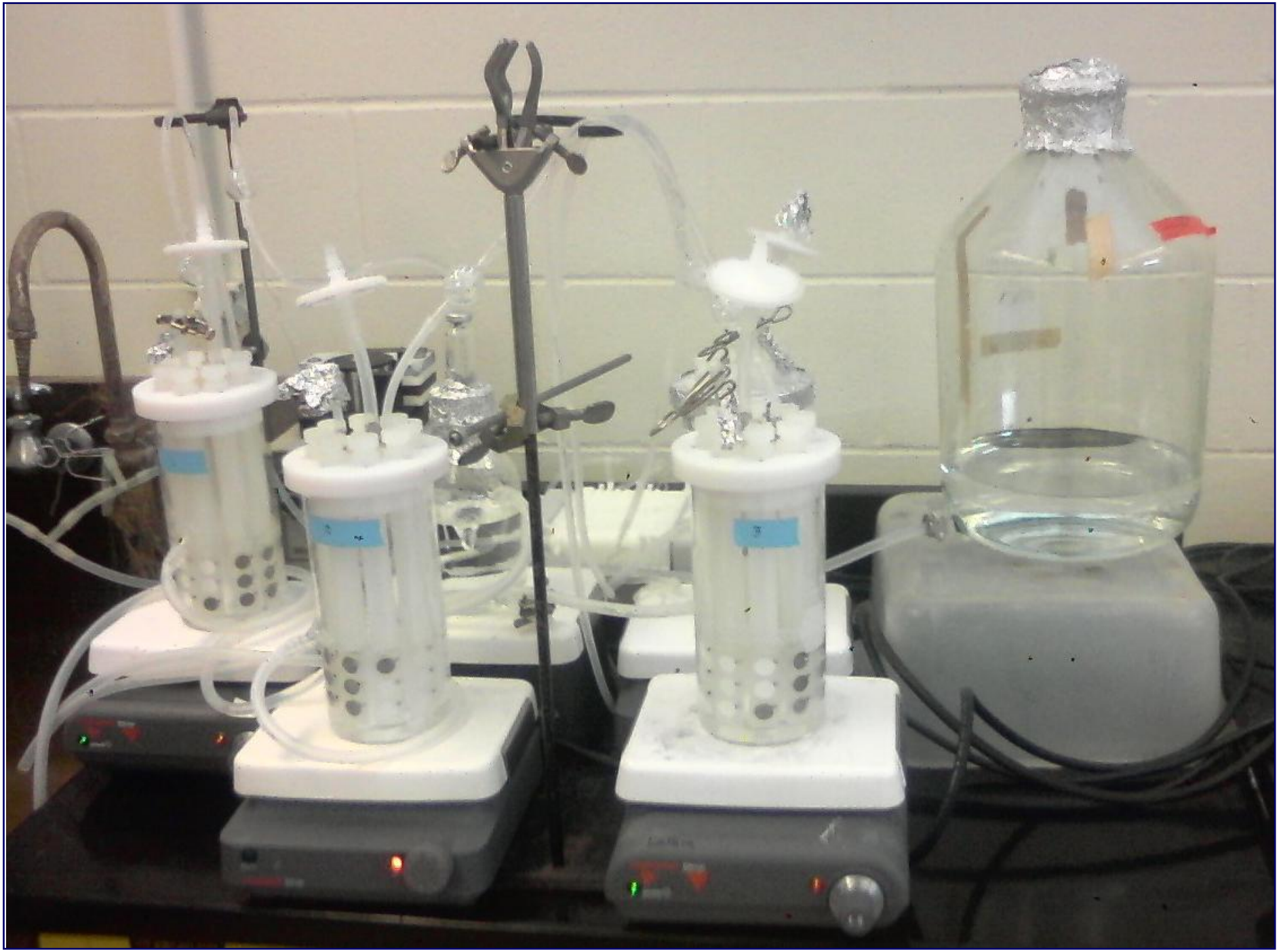
D: Reservoir (Sodium Thiosulfate 1.17 g/L)

E: Pump (0.1 ml/min)

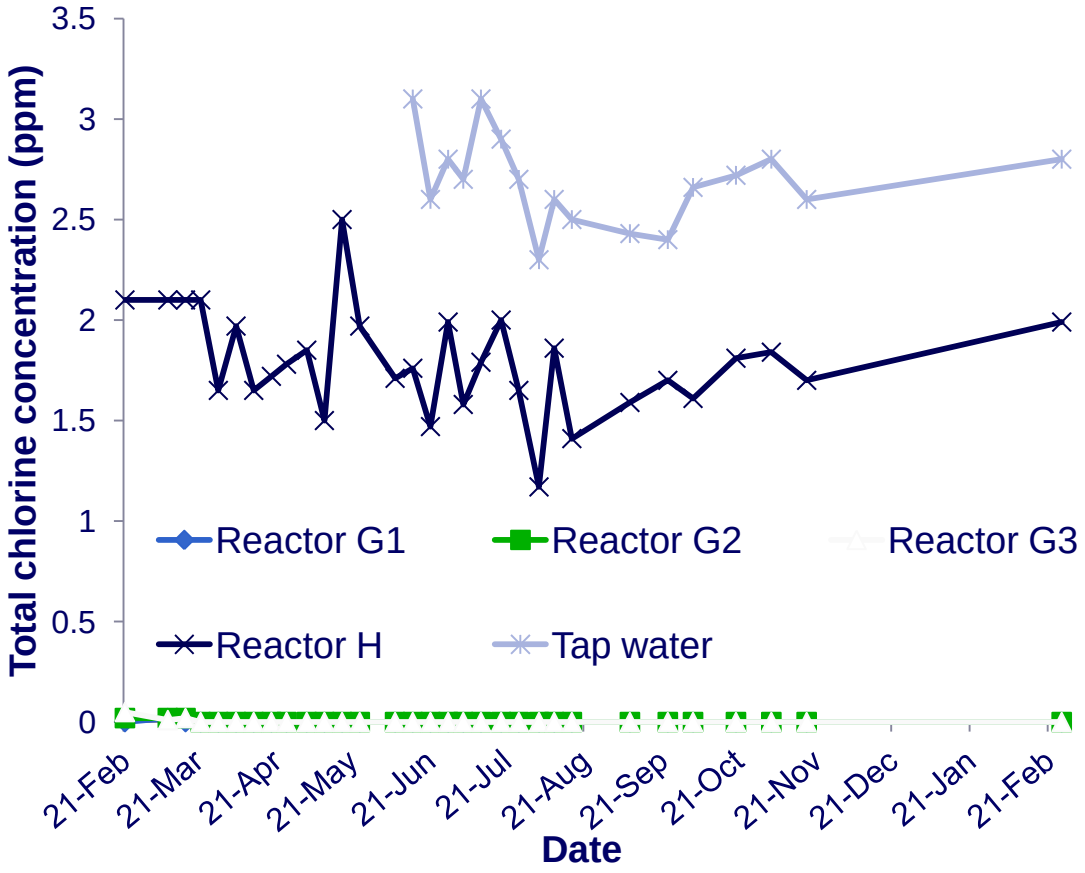
F: Pump (3.9 ml/min)

G1,G2, G3: CDC Biofilm Reactor (125 rpm, dechlorinated tap water)

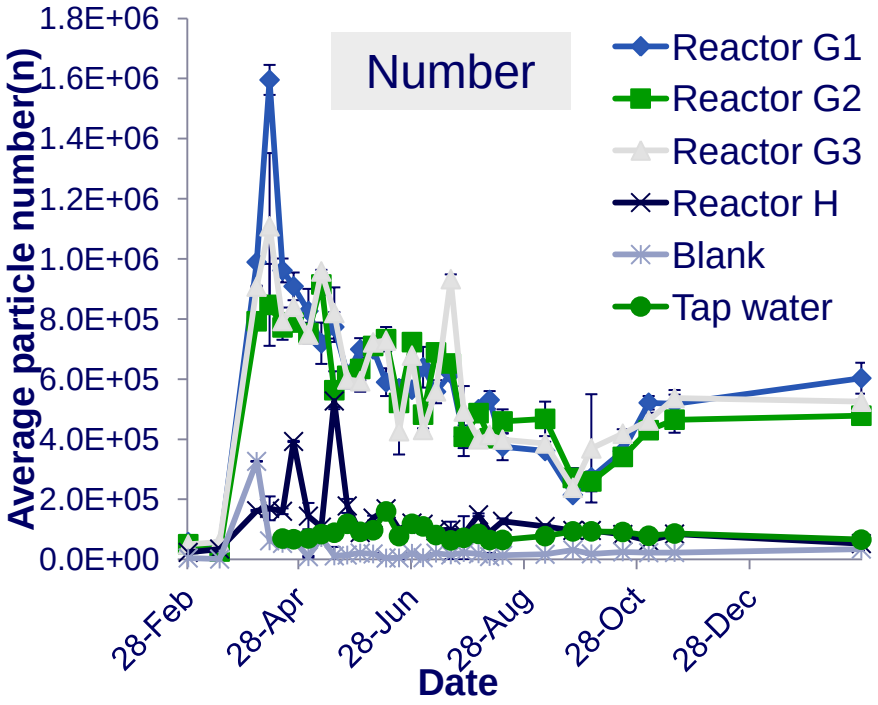
H: CDC Biofilm Reactor (125 rpm, tap water)

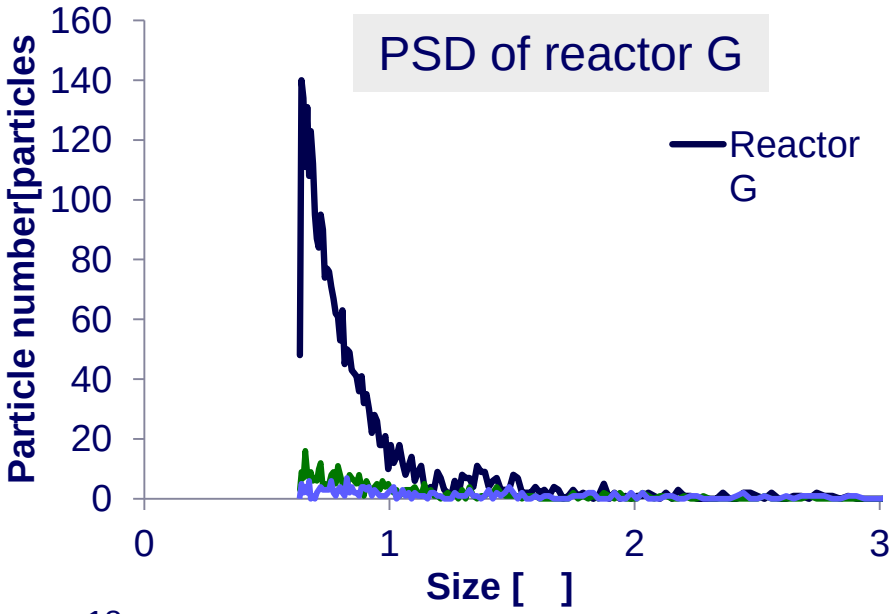


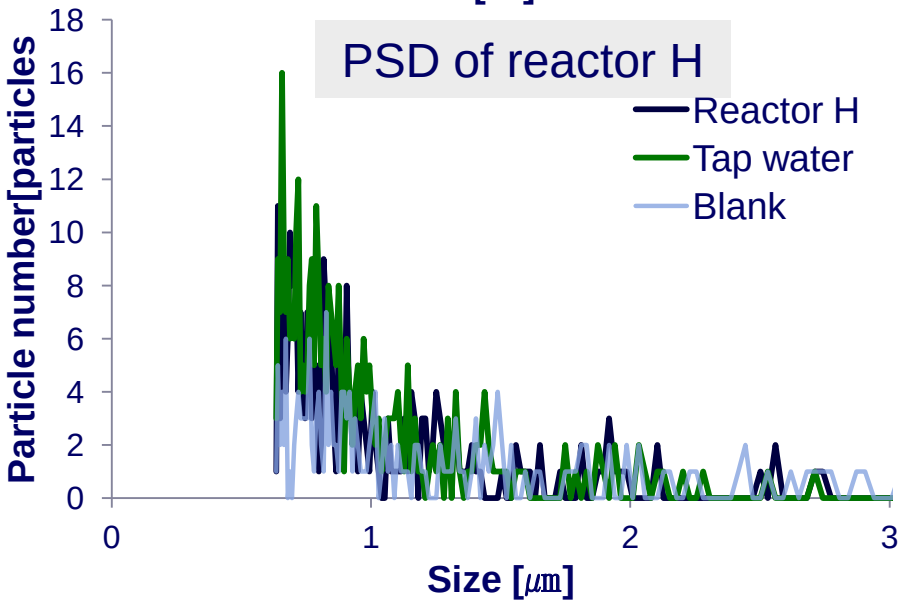
CDC Reactors



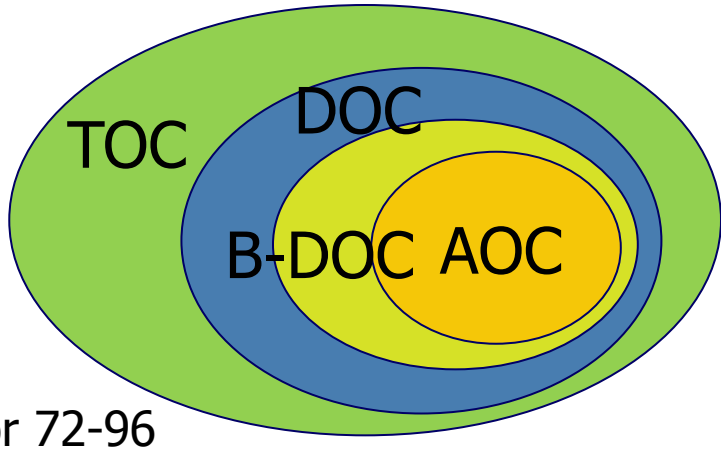
CDC Reactors – *Particle Counter*

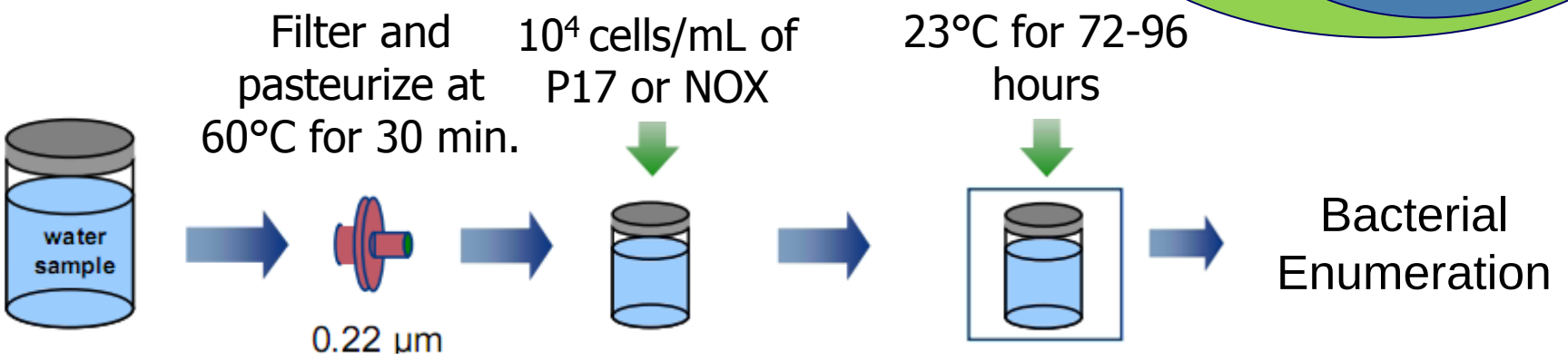






Assimilable Organic Carbon(AOC)





$$AOC\ (\mu g/L) = [AOC_{P17} + AOC_{NOX}]$$

$$AOC\ (\mu g/L) = \left[\frac{\max density\ P17\ (\frac{cfu}{ml})}{yield\ P17\ (\frac{cfu}{\mu g})} + \frac{\max density\ NOX\ (\frac{cfu}{ml})}{yield\ NOX\ (\frac{cfu}{\mu g})}\right] \times 1000(\frac{ml}{L})$$

AOC Method 9217, *Standard Methods*

Expediting the AOC test

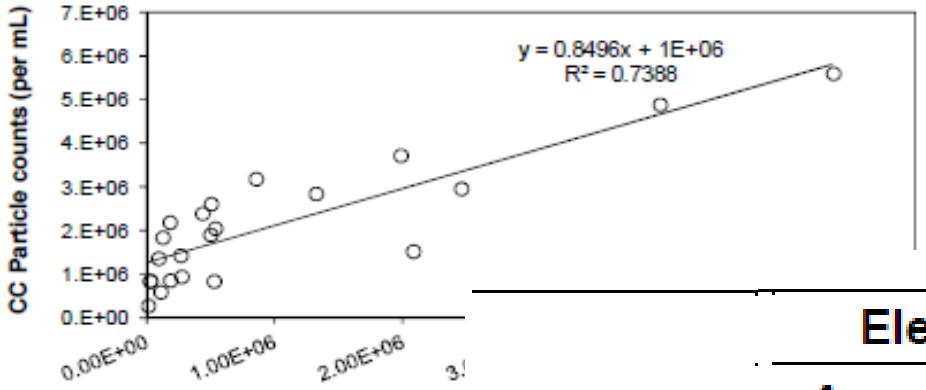
PARTICLE COUNTER

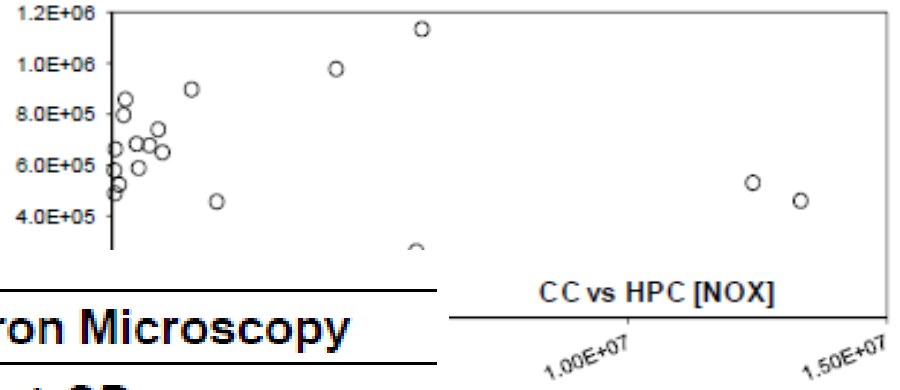
FLOW CYTOMETRY

ATP ASSAY

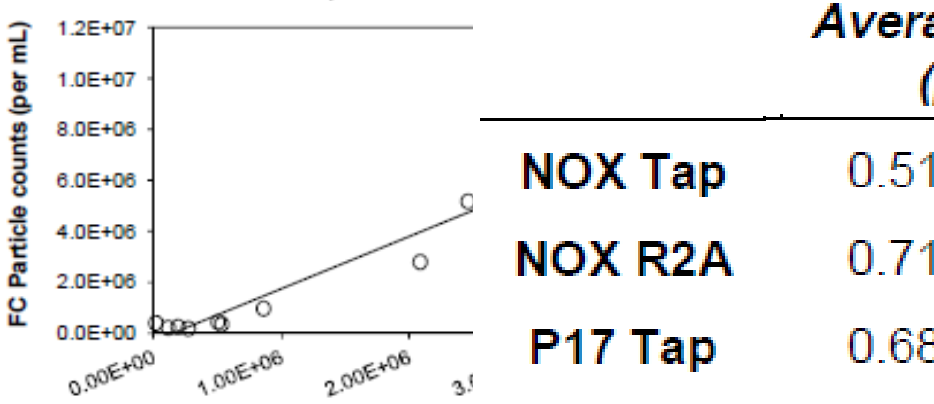
PLATE COUNTS

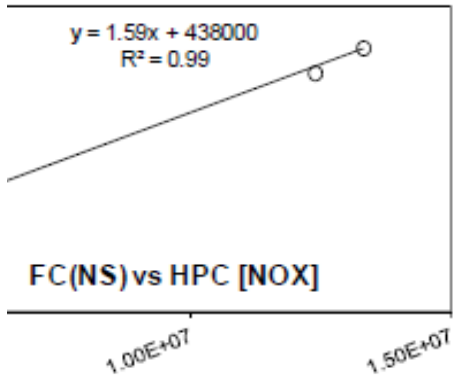
Results (Pooled data)

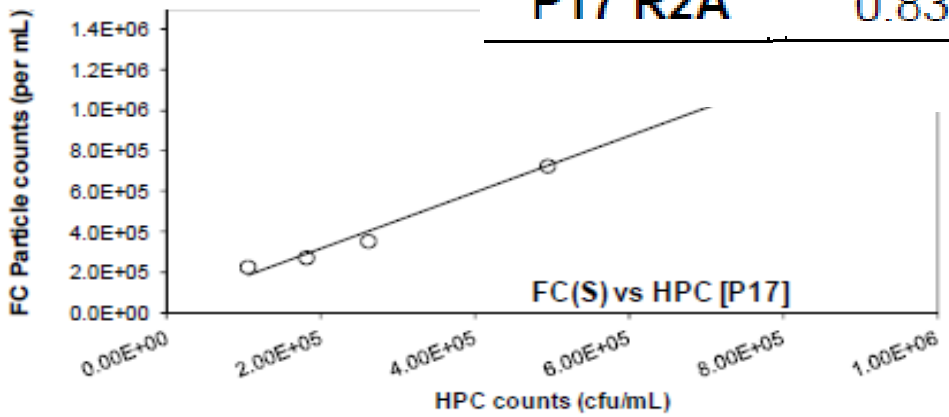


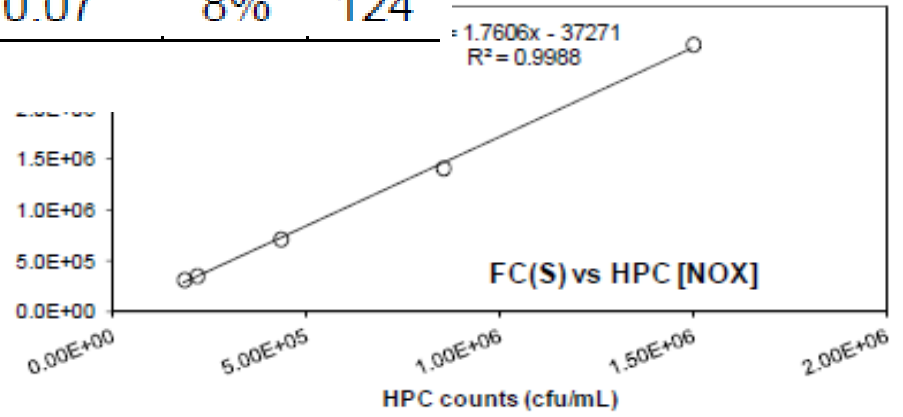


	Electron Microscopy		
	Average ± SD (µm)	*CV*	*n*
NOX Tap	0.51 ± 0.03	7%	68
NOX R2A	0.71 ± 0.05	8%	106
P17 Tap	0.68 ± 0.09	14%	24
P17 R2A	0.83 ± 0.07	8%	124

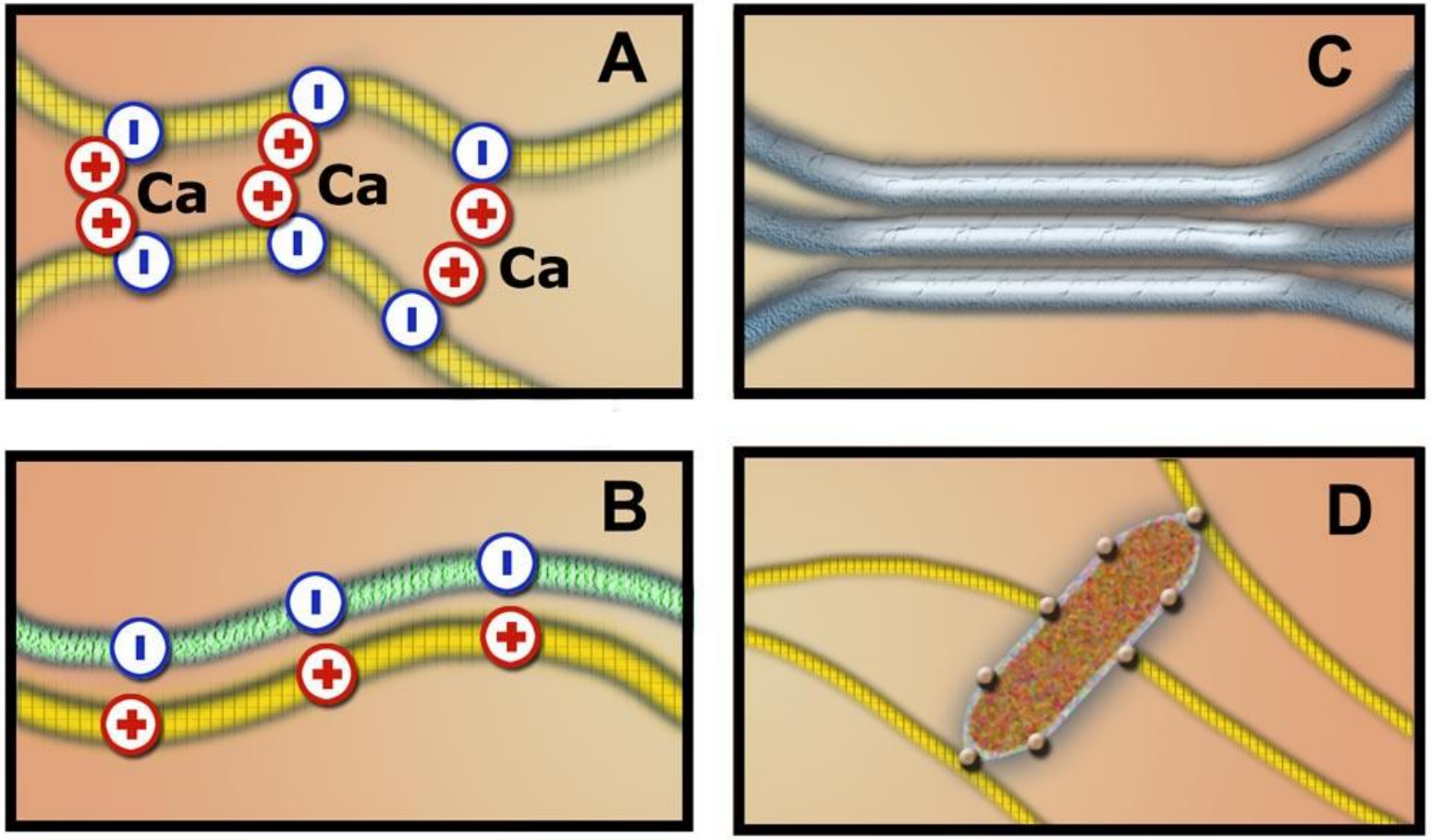








Conceptual Models of Biofilm Cohesion



Courtesy of Phil Stewart at CBE

96 Well Plate Testing

✓ Preparatio n

Culture for 24hrs

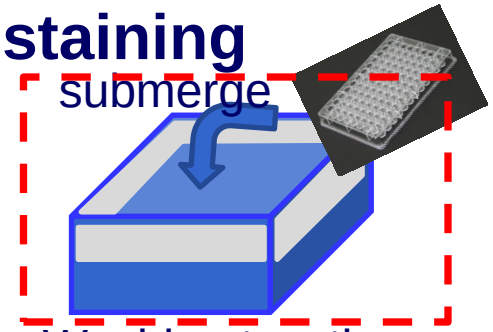
Innoculation

Culture for 24hrs

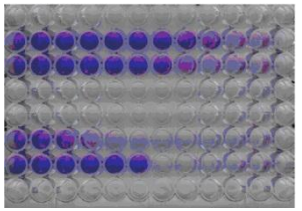
Washing using Milli-Q (use pipet and washed two time

✓ Treatment and staining

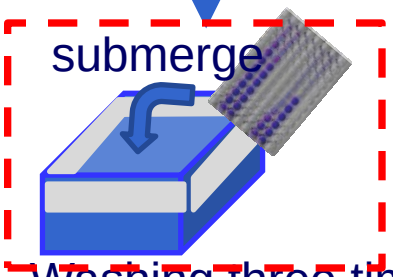
Treatment for 2-24 hrs



Washing two times using tap water



Staining for 15 min using crystal violet

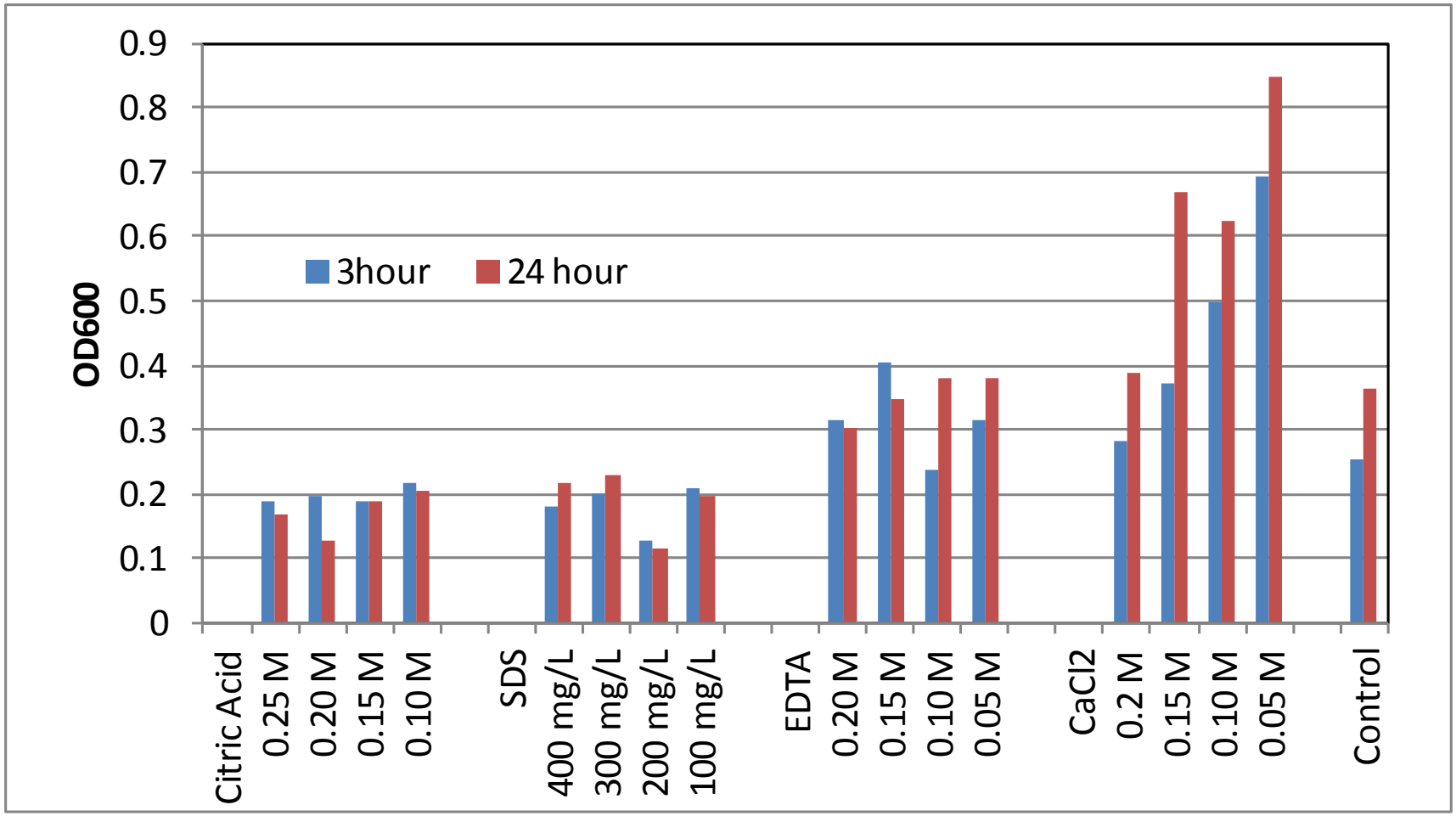


Washing three times using tap water

Add 33% acetic acid

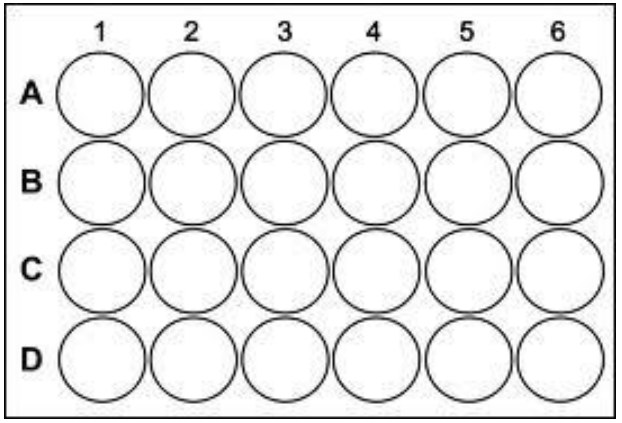
Measure absorbance at OD 600

96 well plates - *S. epidermidis*



24 Well Plate Testing

- Following 6 coupon materials were used
  - Cement
  - Glass
  - PVC
  - HDPE
  - Polycarbonate
  - Polypropylene



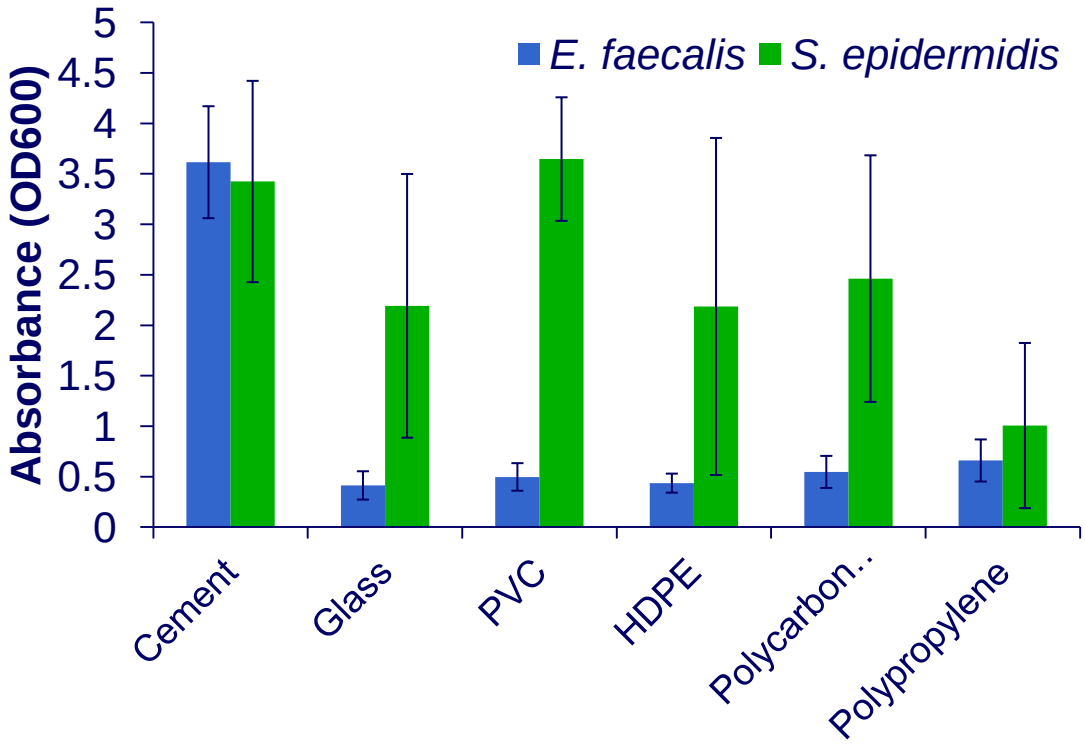
- Following 2 bacterial species were used :
  - *S. epidermidis*
  - *E. faecalis*

Experimental Plan

	1	2	3	4	5	6
A	CE	GL	PV	HD	PC	PP
B	CE	GL	PV	HD	PC	PP
C	CE	GL	PV	HD	PC	PP
D	CE	GL	PV	HD	PC	PP

Cells
Controls

Glossary	
CE	Cement
GL	Glass
PV	PVC
HD	HDPE
PC	Polycarbonate
PP	Polypropylene



Summary

- Higher planktonic growth/activity in dechlorinated reactors
- Flow cytometry a good tool for expediting the current AOC protocol
- Effects of pipe surfaces and treatments on biofilm development/removal being explored using well-plate method
- Roughness matters (cement coupons)

Future Work

- CDC biofilm reactors. Test coupons for:
  - Biomass (total protein, DNA)
  - Thickness, roughness, coverage (CLSM)
  - Community analysis
  - Effect of treatment (weakening, detachment)
- 96 and 24 Well plate tests
  - Exploring polyphosphate and citrate treatments
  - Cultures: *S. epidermidis*, CDC reactor effluent
- Submit paper comparing flow cytometry, particle counting, and HPC for bacterial enumeration in the AOC test